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Determination of Chromium Species in Dietary Supplements Using Speciated Isotope Dilution Mass Spectrometry with Mass Balance

Naudia Martone

Department of Environmental Science and Management, Duquesne University, Pittsburgh, Pennsylvania 15282, United States

G. M. Mizanur Rahman*

Applied Isotope Technologies, Inc., 2403 Sidney Street, Suite 280, Pittsburgh, Pennsylvania 15203, United States

Matt Pamuku

Applied Isotope Technologies, Inc., 2403 Sidney Street, Suite 280, Pittsburgh, Pennsylvania 15203, United States

H. M. Skip Kingston

Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, Pennsylvania 15282, United States

ABSTRACT: In order to determine the health impact of chromium in dietary supplements, the Cr(III) and Cr(VI) must be independently measured and verified with mass balance (sum of both species equaling independent measurements of total chromium), as both may be present in finished products. Because Cr(III) is stable in acidic conditions and Cr(VI) in alkaline conditions, interconversions between species may occur in complex matrices and during analytical extraction, increasing the difficulty of quantification. A study was conducted to determine Cr(VI) and Cr(III) in dietary supplements. EPA Method 3060A extraction protocol was performed to extract Cr(VI), and EPA Method 3052 was performed on the extracted residue to digest the remaining Cr(III). Speciated isotope dilution mass spectrometry (SIDMS), as described in the EPA Method 6800 (update V), was implemented with ion-exchange chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS). Method 6800 uniquely enables tracking and correcting for the bidirectional chromium interspecies conversions that occur during extraction and sample handling prior to instrumental analysis. Mass balance results indicated that the off-the-shelf dietary supplements analyzed during this study contained hexavalent chromium ranging from <DL (detection limit) to 122.4 ± 13.0 μg/g, which corresponds to concentrations from below detection levels to up to 16% of the total chromium content. This type of variation in the final products raises public health issues and points to a need to use a robust method that can accurately and reliably make species measurements including correcting for species conversions.

KEYWORDS: *Cr(VI), speciation, SIDMS, IDMS, microwave extraction, dietary supplements*

INTRODUCTION

Chromium is an element that is stable as trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)] species. Although they are chemical forms or species of the same element, each has a different effect on human health. Cr(III) is considered as an essential trace metal that is found in foods, such as meat and whole grains, typically around 2 μg/serving.[1] An adequate intake value for chromium was established in 2001 by the Institute of Medicine, Food and Nutrition Board and varies with age groups and sex: for 19 to 50 year old adults, 35 μg/day, and women, 25 μg/day.[2] Cr(III) is generally considered essential for insulin, glucose, and lipid metabolism; and its deficiency may be linked to diabetes.[3] Cr(VI), on the other hand, is a known carcinogen that causes lung cancer through inhalation. There is evidence of carcinogenicity and toxicity from ingestion. Less severe effects can include other respiratory reactions, such as coughing and irritation. Dermal and allergic responses, such as nasal perforations and skin rashes, have been reported. Cr(VI) may also cause more severe health problems such as kidney and liver damage.[4]

The health risk from exposure to Cr(VI) has been recognized by national and international organizations. The Occupational Safety and Health Administration (OSHA) has set occupational permissible exposure limits in the air for Cr(VI) at 5 $\mu g/m^3$ over 8 days.[4] The International Agency for Research on Cancer (IARC) has classified Cr(VI) in group 1 as a human carcinogen.[5] Cr(VI) is included in California's Proposition 65 (Safe Drinking Water and Toxic Enforcement Act of 1986) list of chemicals that cause cancer, birth defects, or reproductive problems.[6] Proposition 65 requires businesses to notify the public and take immediate remedial action if one of these

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chemicals, including Cr(VI), is within their products, including dietary supplements.[6]

Because Cr(III) is believed to facilitate metabolism, it is often a component of dietary supplements, particularly multivitamin/multimineral complexes and weight loss formulations. Cr(III) is typically found in different forms, including chelates, chromium picolinate, chromium nicotinate, and chromium chloride. If present and stable in these forms, chromium would be safe for ingestion. However, if there are impurities or there are interconversions during supplement production, conditions may exist that lead to conversion of Cr(III) to Cr(VI) in the final supplement composition. Over the past decade, an increasing number of health emergencies from unsafe formulations, warning letters issued by the FDA for manufacturing violations, and various investigative documents point to the fact that routine and rigorous quality assurance testing has been lacking and, in some instances, not performed. Dietary supplements often contain many other ingredients as well as chromium. Final formulations may consist of a solid or liquid matrix that includes ions, organic material, vitamins, a variety of incipients, and gelatin coating that create a supplement composition that may be neutral or have an influencing electrical potential in solution, be either acidic or alkaline, and which determines the final speciated form of chromium in the finished products sold to the public. Varying product combinations and formulations and changing or unreliable sources of raw materials may all contribute to species content or interconversions, making quality assurance testing for chromium species analysis a specialized and necessary requirement.

The measurement of total chromium content alone will not provide appropriate information to make an assessment about health risks or benefits if the concentration of total chromium is above the safety threshold limit. Therefore, it is critical to quantify both Cr(III) and Cr(VI) using a specialized analytical protocol and verify the accuracy of the results through mass balance. The necessary analytical measurement is the determination of the Cr(III) and Cr(VI) concentration values before starting sample preparation in the raw materials and then in the final product. This way, the values would reflect the original concentrations for both chromium species in the original sample matrix and the final form of the supplement sold to the public as required for both labeling laws and other statutes. The highly reactive characteristics and varying stabilities of these two major chromium species render traditional external calibration curve quantification ineffective and inappropriate because it cannot account for and correct for many analytical and interspecies conversion errors. Calibration curve results are not suitable for reliable accuracy, precision, and legally defensible quantification when active species are being analyzed.[7]

The bidirectional interconversions between Cr(III) and Cr(VI) can occur at every step of sample preparation, even while samples in vials wait in the autosampler and during analyses. The direction and level of conversion are primarily influenced by the stability of each chromium species at different pH and Eh values and the matrix surrounding the chromium species. Cr(III) is stable at lower pH and Eh, while Cr(VI) is stable at higher pH and Eh. The lower the pH of a solution, the more likely it is for the species of chromium to be Cr(III) or be converted to Cr(III). Therefore, if sample preparation involves using an acid, Cr(VI) present in the original medium could be significantly converted to Cr(III) because of the extraction conditions. Since it is impossible to keep both chromium species in stable forms prior to analysis, the ability to track and correct for these interconversions is essential during sample preparation to obtain accurate data.

Current analysis of chromium often involves only the determination of total chromium or only Cr(VI) using traditional calibration curve methods, which can result in 40% or wider biases and non-legally defensible results.[7] The EPA Method 7196A is one of the two methods for Cr(VI) determination.[8] It utilizes ultraviolet−visible spectrophotometry for the determination of Cr(VI), which forms a violet color when mixed with a colorimetric reagent. The EPA Method 7199 is another method used for the determination of Cr(VI) in drinking water, groundwater, and wastewater.[9] It also relies on detection of the absorption of a violet color representative of Cr(VI). Method 7199 is specified for water samples, not for the analyses of dietary supplements. Both methods are unsuitable because they do not provide a way to measure Cr(III) and Cr(VI) and achieve mass balance. Furthermore, neither of these methods allows for tracking of interconversions.

One of the goals of this research was to apply four different EPA sample preparation and analysis methods (EPA Method 3060A, EPA Method 3052, EPA Method 7196A, and EPA Method 6800) to the same sample to determine Cr(VI) and Cr(III) and total chromium concentrations.[8,10−12] EPA Method 3060A is an alkaline digestion procedure that extracts selectively Cr(VI) from soils, solids, and similar materials. EPA Method 3052 is a microwave enhanced chemical acid digestion procedure that completely decomposes the sample matrices and brings the analyte of interest into the solution phase as total element. These methods involve extractions and digestions both performed in a laboratory microwave system. The process allows the species to be available in a solution. A chemically inert ion-exchange chromatograph (IC) can then be used to separate the two species, which are then detected by an inductively coupled plasma mass spectrometer (ICP-MS) interfaced with the IC. These instruments and methods facilitate streamlined, high-sensitivity detection. EPA Method 6800, speciated isotope dilution mass spectrometry (SIDMS), which facilitates tracking and correction between the two chromium species, was implemented as the benchmark EPA method for accurate Cr(VI) analysis (update V).[12] The fundamental and applicable theory of SIDMS in environmental human health systems is established and documented.[12−24]

MATERIALS AND METHODS

Specific Samples and Sample Handling. Eleven (11) unknown chromium supplement powder specimens were received from ConsumerLab.com, inventoried, checked against the sample log, and stored at room temperature in a clean room cabinet until further processing. Thirteen (13) additional samples comprising tablets, caplets, capsules, and liquid mixture were off-the-shelf products purchased from local retail outlets in Pittsburgh, Pennsylvania, USA. Specimen containers were labeled with codes that were used for individual tracking. Unless noted otherwise, all analytical manipulations were conducted in the class-1000 clean room or on the class-100 clean bench, as appropriate.

Instrumentation. A laboratory microwave system (Ethos 1: *Advanced microwave digestion system* from Milestone, Inc., Shelton, CT, USA), equipped with temperature and pressure feedback control and magnetic stirring capability was used in this study. This device is accurate in temperature sensing and control to within ±2.0 °C of set temperatures, and it automatically adjusts the microwave field output power to achieve preset temperatures. The high-pressure rotor was used with ten simultaneous extraction vessels per batch. The high-

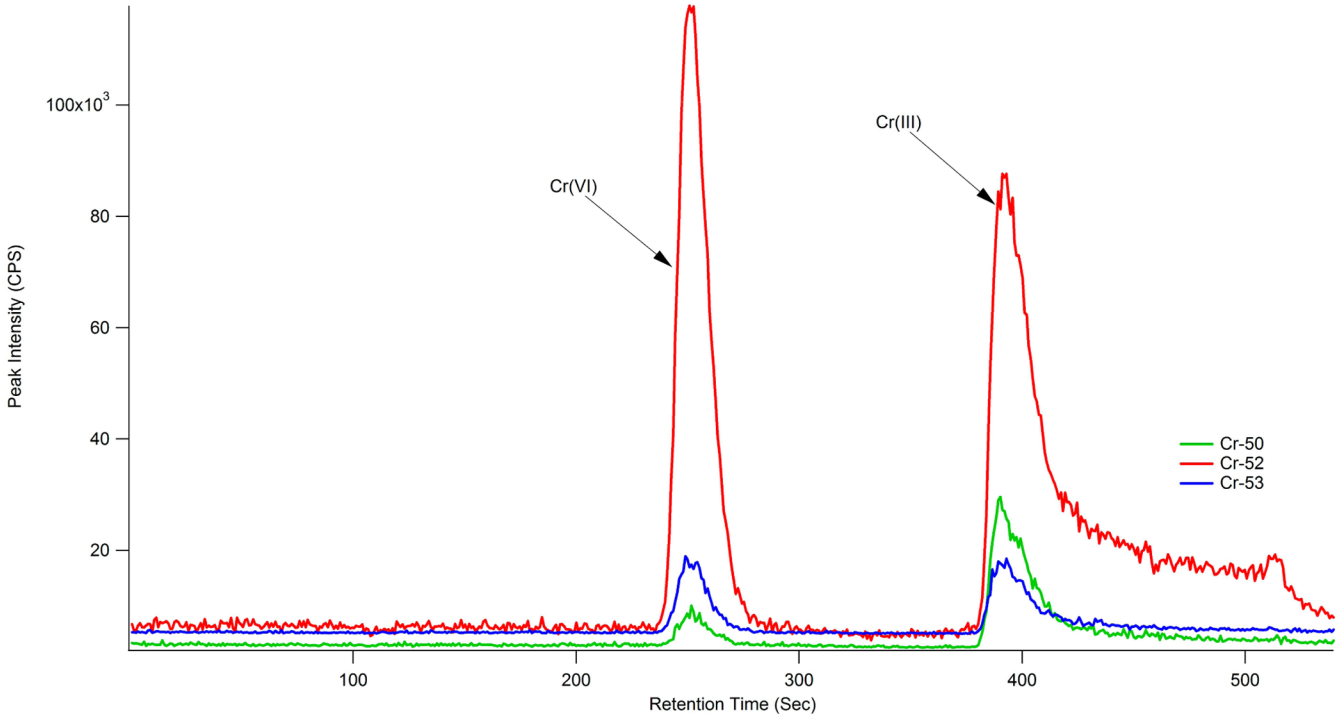

Figure 1. IC-ICP-MS chromatogram for 25 ppb Cr(III) and Cr(VI) standard. Flow rate: 1.0 mL/min with gradient elution. Eluent: A = 0.06 M HNO_3, pH = 9.3; B = 0.06 M HNO_3, pH 1.2. Column: PRP-X100 anion exchange (150 mm × 4.6 mm, 10 μm), from Hamilton.

pressure (maximum operating pressure and temperature: 100 bar and 300 °C) closed digestion vessels (model SK10, Milestone, Inc., Shelton, CT, USA) used for both digestions and extractions are made of high purity TFM (a thermally resistant form of fluoropolymer) and have a capacity of 100 mL each. A FAM-40 vacuum unit (Milestone, Inc., Sorisole (BG), Italy) was used to filter the digests and extracts. An Analytical *Plus* Electronic Balance (OHAUS, England), capable of weighing 0.00001 g, was used in this study to weigh samples, reagents, and standards.

The chromatography system (Metrohm USA, Riverview, FL) consisted of two 818 IC pumps, a 762 IC interface, software IC Net v2.3 SR3, and an 838 IC autosampler. The separation system included a six-port sample injector equipped with a 100 μL sample loop. An anion-exchange column, PEEK 150 mm × 4.6 mm, 10 μm (PRP X-100, Hamilton) was used in this study to separate Cr(VI) and Cr(III). Because no special interface is required between the PRP X-100 Cr column and the ICP-MS (Agilent 7700, Agilent Technologies, Santa Clara, CA, USA], one outlet of the column is directly interfaced to the nebulizer of the ICP-MS with a piece of PEEK tubing, and the other end is connected to a 100 μL sample loop. The sample delivery system consisted of peristaltic pump, micromist nebulizer, quartz spray chamber, and quartz torch. The instrument was fitted with nickel sampler and skimmer cones and optimized daily using 1 ppb tuning solution (Agilent Technologies, Santa Clara, CA, USA) containing Li, Y, Ce, and Tl in 1% HNO_3. Figure 1 shows a typical separation of Cr(III) and Cr(VI) using this system at a flow rate of 1.0 mL/min. The operating conditions of the IC-ICP-MS are shown in Table 1.

Instrument grade liquid argon (Airgas Inc., Radnor, PA, USA) was used as the nebulizer carrier gas. Polypropylene graduated 50 mL centrifuge tubes were used as extract and digest storage vials. Standard stocks were prepared and stored in Teflon bottles. Polystyrene dilution vials with HDPE snap cap were used for preparation of all working sample solutions.

Reagents and Materials. Water for dilutions and other makeup volume activity was 18 MΩ, double deionized (DDI) water derived from a Barnstead NANOpure Ultrapure water purification system (Dubuque, IA, USA). Concentrated HNO_3 (16 mol/L) (*TraceMetal*, Fisher Scientific, Pittsburgh, PA, USA) and concentrated H_2O_2 (30%) (Fisher Scientific) were used to prepare the reagent medium for total chromium digestion. Concentrated NH_4OH (aq) (15 mol/L) was prepared by bubbling high purity ammonia gas through DDI water. Extraction medium for Cr(VI) containing 0.5 mol/L NaOH and 0.28 mol/L Na_2CO_3 was prepared by dissolving 20.0 g of NaOH (98.0%) (Certified ACS, Fisher Scientific) and 30.0 g of Na_2CO_3 (99.6%, Certified ACS, Fisher Scientific) in 1000 mL of DDI water. 1 mol/L phosphate buffer (pH = 7) was prepared by dissolving 87.09 g of K_2HPO_4 and 68.04 g of KH_2PO_4 into 1000 mL of DDI water, as described in the US EPA Method 3060A.[11]

Four standard stock solutions, ^{nat}Cr(III) standard (10 μg/g in 1% HNO_3), ^{nat}Cr(VI) standard (10 μg/g in DDI water), ^{50}Cr(III) spike, 128 μg/g in 1% HNO_3, and ^{53}Cr(VI) spike, 90 μg/g in 0.5% NH_4OH, were obtained from Applied Isotope Technologies, Inc. (Pittsburgh, PA, USA). The "nat" superscript stands for "natural isotopic

Table 1. Chromatographic and ICP-MS Operating Conditions for Cr(III) and Cr(VI) Speciation

IC Conditions	
LC pump	Metrohm 818 IC pumps (PEEK)
column	PRP X-100 anion exchange column (150 mm × 4.6 mm, 10 μm) (PEEK) (Hamilton)
mobile phase	A, 0.06 mol/L NO_3^- (pH 9.3 adjusted with NH_4OH); B, 0.06 mol/L NO_3^- (pH 1.2)
flow rate	1 mL/min
gradient program	(1) 0 min, 0% B; (2) 3.0 min, 0% B; (3) 3.1 min, 100% B; (4) 7.0 min, 100% B; (5) 7.1 min, 0% B; (6) 9 min, 0% B
injection volume	100 μL
column temperature	ambient
ICP-MS Conditions	
Micromist Nebulizer with Quartz Spray Chamber	
monitoring isotopes	^{50}Cr, ^{52}Cr, and ^{53}Cr
acquisition mode	time resolved analysis
integration time per mass, s	0.1
total analysis time	9 min
collision cell gas	He (4.5 mL/min)

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abundance". The isotope abundances of natural and isotope-enriched chromium materials used in this study are reported in Table 2. The preparation procedure for each standard and characterization of standard solutions can be found in refs 12−15.

Table 2. Isotope Abundances for Chromium Standards

isotope	^{Nat}Cr (%)	^{50}Cr(III)	^{53}Cr(VI)
^{50}Cr	4.345	97.300	0.030
^{52}Cr	83.789	2.400	2.190
^{53}Cr	9.501	0.200	97.700
^{54}Cr	2.365	0.100	0.080
total	100.000	100.000	100.000

The IC eluent was prepared by mixing 7.8 mL of concentrated HNO_3 (*Trace metal*, Fisher Scientific, Pittsburgh, PA, USA) and 2.0 mL of 10,000 μg/mL thulium (Tm) standard (High Purity Standards, Inc., Charleston, SC, USA) in a 2 L polyethylene bottle. The final pH of eluent A and eluent B was 9.3 and 1.2 containing 10 ppm thulium for optimum separation of Cr(III) and Cr(VI) on the column. The pH of eluent A was adjusted to 9.3 by adding concentrated NH_4OH. The working solutions of ^{nat}Cr(VI) and ^{nat}Cr(III) were prepared daily by weighing proper amount of stock ^{nat}Cr(VI) and ^{nat}Cr(III) and diluting with DDI water to the desired mass. Chromium working solutions (0, 10, 20, 30, 40, 50, and 60 ppb) for deadtime and mass bias correction were prepared daily by measuring proper amount of stock ^{nat}Cr standards and diluting with DDI water to the desired mass.

Sample Preparation Procedures. *Total Chromium by Method 6800 IDMS.* All samples were digested for total chromium according to the US EPA Method 3052 (*Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices*).[10] A closed vessel microwave system with automatic temperature control and continuous stirring was used as a heating device. Approximately 0.50 g of a representative specimen was weighed into a microwave vessel and combined with 2 mL of H_2O_2 and 9 mL of 16 mol/L HNO_3. Then, a known amount of isotopically enriched trivalent chromium [^{50}Cr(III)] was added into each vessel along with a magnetic stir bar for homogeneous mixing. Vessels were sealed and microwave irradiated at 180 ± 5 °C for 10 min, following a 10 min ramp to 180 °C. After cooling to ambient temperature, the sample digests were filtered through a 0.45 μm glass fiber filter and stored in a cold-room at 4 °C until analyzed (usually less than two days). Each microwave digestion run was composed of ten samples, one of which was a reagent blank. Each digested sample was diluted by a factor of ∼50 with 0.5% HNO_3. In order to determine the total chromium concentration in each sample, the digests were analyzed by ICP-MS in spectrum mode and the concentration was calculated by IDMS software.[12]

Hexavalent Chromium with Methods 3060A and Method 6800 SIDMS. The sample preparation for Cr(VI) alkaline extraction follows the protocol of the US EPA Method 3060A. A closed vessel microwave system with automatic temperature control and continuous stirring was used as a heating device. Approximately 0.50 g of food supplement specimen, proper amounts of ^{50}Cr(III) *and* ^{53}Cr(VI) (*double-spiking*) were weighed into a microwave vessel. Also, 24.5 mL of the extraction solvent along with 50 mg of $MgCl_2$ and 0.5 mL of 1 mol/L phosphate buffer was added to the vessel. To each sample before extraction analytically appropriate amounts of ^{50}Cr(III) and ^{53}Cr(VI) were added, to result in a ratio of measured isotope that was close to the optimum isotope ratio for each species of interest. Optimization spiking factors were calculated for each isotope enrichment and each natural isotope combination.[25] The amount of isotopic spike depends on the expected levels of Cr(VI) and Cr(III) in the sample, the enrichment purity of the reagent isotope, and the natural ratio of that corresponding isotope as they occur in nature. The optimum spike is achieved by knowing the aforementioned values and for this standard by obtaining the certified value of each species from the certificate of analysis, or in an unknown with prior workup of unspiked sample to estimate the approximate concentration of each species present in the sample. The optimum amount of isotopic analogue that has to be spiked to obtain more accurate and precise measurement of both Cr(III) and Cr(VI) performing IDMS and/or SIDMS analysis is spread over 2 orders of magnitude, i.e., the sample to spike ratio needs to be between 0.1 and 10. During this study, an optimum isotope ratio of approximately 1:1 (sample:spike) was used. Three replicates of each specimen were extracted for this study. Vessels were sealed and heated at 95 ± 2 °C for one hour, after a ten minute ramp to 95 °C. After cooling to room temperature, the solutions were filtered using a 0.45 μm Millipore glass fiber filter (Fisher Scientific, Pittsburgh, PA). The filtered extracts were stored in a cold-room at 4 °C until analyzed. A 50 ng/g mixed calibration standard for determining mass bias correction was prepared from natural abundant Cr(VI) and Cr(III) primary standard stock. The mass bias standard was analyzed at the beginning, middle, and end of each sample analysis sequence. The algorithms, assumptions, and detailed SIDMS calculations are discussed elsewhere.[12−24]

Hexavalent Chromium with Method 3060A and Method 7196A. The widely used EPA Method 7196A was performed on some selected raw material samples (pure chromium compounds that are being used for dietary supplement production by the manufacturers) after extracting Cr(VI) using Method 3060A. Method 7196A involved the preparation of DPC solution by adding 250 mg of DPC (Certified ACS, Fisher Scientific) in 50 mL of acetone in a brown bottle. A

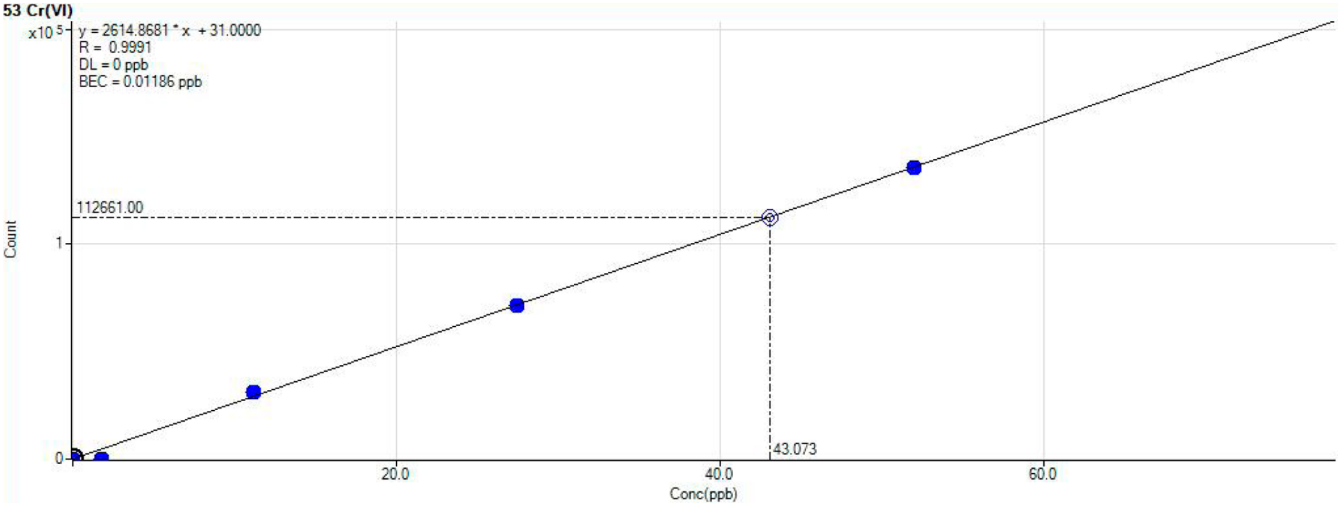

Figure 2. Calibration curve for Cr(VI).

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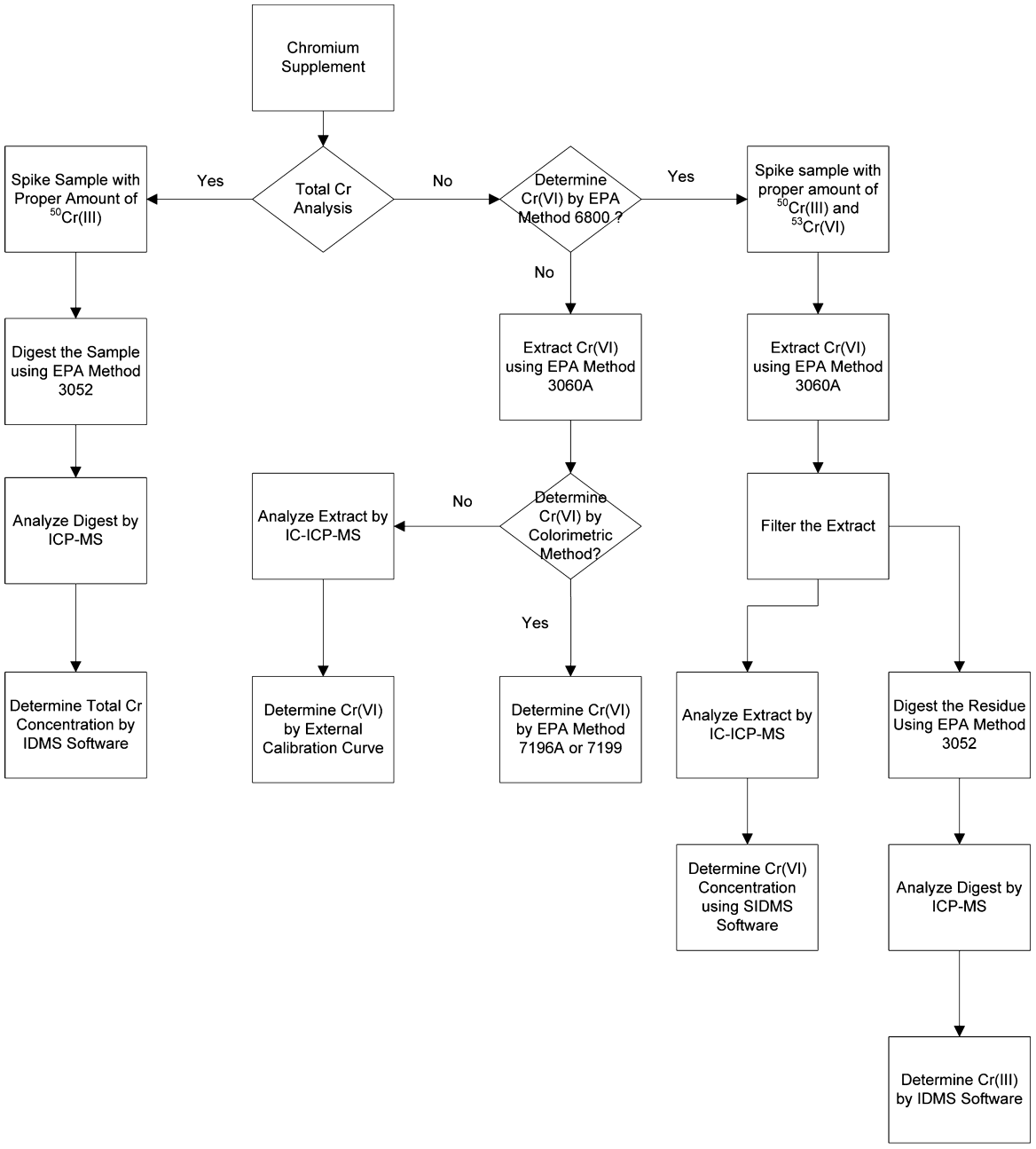

Figure 3. Flowchart of the experimental process for chromium speciation in dietary supplements.

solution of 10% sulfuric acid was also prepared with 10 mL of DDI water and 1.0 mL of concentrated sulfuric acid (Trace Metal, Fisher Scientific). The DPC and acid solution were added to a vial along with the sample extract. Since this is a light sensitive reaction that causes the color change, the analysis was performed shortly following the reaction. A Cary 100 E UV visible spectrophotometer was used to measure the absorbance of the violet color, indicative of Cr(VI), at 540 nm. A calibration curve with hexavalent chromium standards (0, 25, 50, 100, and 200 ppb) was created to determine the Cr(VI) concentrations in the samples.

Development of and Application of Mass Balance. In order to perform mass balance on the specimens, different extraction procedures were evaluated for the extraction of both Cr(III) and Cr(VI) in the same solution. The extraction solvents that were initially tested during this method development study contain hot water, alkaline solution containing ethylenediamine tetraacetic acid (EDTA),[26] and phosphate buffer. None of the procedures were optimal. Therefore, the EPA Method 3060A was chosen for sample preparation during mass balance study. Initially the Cr(VI) was extracted using the alkaline solution and then the sample residue was digested using EPA Method 3052 for Cr(III) analysis. In order to perform mass balance on the samples, the extracts and digests were analyzed with ICP-MS and IC-ICP-MS, respectively, by using two sets of calibration standards. One set of calibration standards were prepared by matrix matching for trivalent chromium in acidic digests. The second set of calibration standards were prepared also by matrix matching for a mixture of Cr(VI) and Cr(III) (if any) in alkaline extracts (Figure 2). During this study, two samples were chosen based on the conversion profile from SIDMS study. One sample was chosen from the lower conversion side [sample 9, 2.4% conversion from Cr(III) to Cr(VI)], and the other sample was from the higher conversion side [sample 12, 47.9% conversion from Cr(III) to Cr(VI)]. For SIDMS analysis, the sample was spiked with isotopically enriched hexavalent chromium and the Cr(VI) extracted from the sample by using the microwave version of EPA Method 3060A thereby stabilizing the Cr(VI). During this protocol, Cr(III) precipitates out of

Table 3. Analysis of Total Chromium in Dietary Supplements by Isotope Dilution Mass Spectrometry (IDMS, EPA Method 6800)[a]

sample ID	unit type	claimed amount of total Cr/unit (μg)	av unit wt (g)	av total Cr concn (μg/g)	total Cr measd by IDMS/g sample (μg/g)	total Cr measd by IDMS/unit (μg)	% difference from reported total Cr/unit value
1	tablet	190	0.269	706.3	777.1 ± 4.6	209 ± 1	+10
2	capsule	210	0.283	742.0	818.0 ± 10.9	231 ± 3	+10
3	caplet	175	1.083	161.6	209.6 ± 5.0	227 ± 5	+30
4	caplet	70	1.397	50.1	41.6 ± 3.2	58 ± 4	−17
5	caplet	70	1.350	51.9	55.1 ± 1.1	74 ± 1	+6
6	caplet	225	0.866	259.8	240.9 ± 2.2	209 ± 2	−7
7	tablet	210	1.159	181.2	170.5 ± 1.9	198 ± 2	−6
8	capsule	195	0.582	335.1	331.8 ± 19.2	193 ± 11	−1
9	tablet	525	0.446	1,177.1	1,095.9 ± 21.5	489 ± 10	−7
10	tablet	210	0.432	486.1	510.8 ± 7.0	221 ± 3	+5
11	capsule	190	0.402	472.6	674.9 ± 15.1	271 ± 6	+43
12	caplet	200	1.044	191.6	205.5 ± 2.9	215 ± 3	+8
13	caplet	200	1.040	192.3	229.4 ± 4.6	239 ± 5	+20
14	caplet	200	0.866	230.9	302.8 ± 28.1	262 ± 24	+31
15	caplet	250	1.132	220.8	243.3 ± 4.0	275.4 ± 5	+10
16	tablet	200	1.145	174.7	187.5 ± 7.7	215 ± 9	+8
17	tablet	200	0.439	455.6	548.3 ± 5.6	241 ± 2	+20
18	liquid	60	32.785	1.83	3.09 ± 0.03	93 ± 1	+55
19	tablet	500	0.435	1,149	561.1 ± 11.2	244 ± 5	−51
20	capsule	200	0.484	413	860.9 ± 5.6	375 ± 2	+88
21	caplet	66.5	1.344	49.5	58.9 ± 1.6	79 ± 2	+19
22	caplets	66.5	1.411	47.1	47.1 ± 1.3	67 ± 2	+1
23	capsule	200	0.603	331.7	384.1 ± 11.5	231.6 ± 6.9	+16
24	tablet	6.7	0.649	10.3	11.9 ± 0.8	7.7 ± 0.5	+15

[a]Uncertainties are at 95% CL with $n = 3$.

solution and remains in the solid phase. After extraction with Method 3060A and filtration or high speed centrifugation, the supernatant was decanted and analyzed for Cr(VI) by IC-ICP-MS. Then, the residues from the previous extraction step were digested after spiking with isotopically enriched trivalent chromium and using Method 3052 for analysis of Cr(III) by ICP-MS. After evaluation of the results from the study, it was found that this procedure (selective extraction and digestion), when applied for supplement analysis, does not correct for the conversion of Cr(III) to Cr(VI) during alkaline extraction. At this point, it was decided to use the traditional alkaline extraction method (EPA Method 3060A) using double spike with ^{50}Cr(III) and ^{53}Cr(VI) before extraction. After the selective extraction of Cr(VI) from the sample, the residue was digested using Method 3052 for the determination of Cr(III) that was precipitated out during alkaline extraction of Cr(VI). The alkaline extract was analyzed for Cr(VI) and the acidic digest was analyzed for Cr(III) by IC-ICP-MS. A schematic diagram of the entire sample preparation and analysis processes is shown in Figure 3.

RESULTS AND DISCUSSION

Quantification Methods. Two quantification methods described in EPA Method 6800 were used for chromium species analyses throughout this study: (1) IDMS for total chromium, and (2) SIDMS for Cr(VI), where Cr(III) precipitates out of solution during alkaline extraction. EPA Method 7196A was also used to analyze hexavalent chromium. In its optimized and robust form, SIDMS facilitates simultaneous accounting of both Cr species concentrations and correction for transformations by enabling isotopic tagging for the oxidation of Cr(III) and the reduction of Cr(VI) during analysis in aqueous samples. Thus, the combined extraction/SIDMS procedure is capable of correcting for bidirectional species transformations that may occur during analyses of Cr(VI) in solid samples. Another important point to make is that IDMS and SIDMS methods will uniquely and reliably make these corrections which are expected to occur on a sample-to-sample basis, considering the highly dynamic nature of these species and other factors, such as the analysts' tendencies, procedural variations, partial analyte recovery, and even instrument detector drift. The advantages of EPA Method 6800 can only be realized by using direct mathematical algorithms and correction calculations. Similar level of quantification, data, and statistical robustness cannot be achieved by traditional calibration curve implementations.

Total Chromium Analysis by IDMS. The samples were digested according to EPA Method 3052 and were analyzed with ICP-MS in spectrum mode. The concentrations of total Cr were determined by using Cr-SPC software (Applied Isotope Technologies, Inc., Pittsburgh, PA, USA, www.sidms.com) which was supplied as a kit along with the isotopically enriched Cr(III) and Cr(VI) standards. Results are summarized in Table 3 column 6. Total elemental analysis provides an evaluation of the sample matrix. From Table 3, it is seen that sample 9 contains a higher amount of total chromium (1,095.9 ± 21.5 μg/g) compared to sample 18 (3.09 ± 0.03 μg/g). The total chromium values were measured in μg/g unit, converted to total chromium values per unit of sample, and then compared with the manufacturer's tabulated value. In general, the total chromium values obtained by the IDMS protocol were within ±20% of the reported values on the supplement packages in eighteen out of the twenty-four specimens studied. However, some values are noticeably different, (e.g., samples 19 and 20) from those reported on the supplement package. The reported value for sample 19 is nearly double the total chromium concentration measured by IDMS in the specimen. On the other hand, the reported value for sample 20 is approximately

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Table 4. Mass Balance Study for Trivalent and Hexavalent Chromium in Dietary Supplements Using SIDMS Protocol[a]

			mass balance study using SIDMS and IDMS (μg/g)				
sample ID	pH/Eh (mV)	total Cr by IDMS (μg/g)	trivalent Cr (IDMS)	hexavalent Cr (SIDMS)	total Cr (sum of trivalent and hexavalent)	Cr(III) to Cr(VI) conversn (%)	Cr(VI) to total Cr (%)
1	6.7 (−2.4)	777.1 ± 4.6	692.0 ± 20.2	26.4 ± 2.5	718.4 ± 20.4	0.5 ± 0.2	3.4
2	5.2 (94.5)	818.0 ± 10.9	790.5 ± 8.2	<DL	790.5 ± 8.2	0.3 ± 0.1	
3	5.4 (78.0)	209.6 ± 5.0	720.8 ± 47.1	17.0 ± 2.3	737.8 ± 47.2	39.6 ± 6.4	8.1
4	6.3 (25.8)	41.6 ± 3.2	31.3 ± 1.8	2.6 ± 0.5	33.9 ± 1.9	6.6 ± 0.9	6.3
5	6.3 (22.6)	55.1 ± 1.1	58.5 ± 1.4	<DL*	58.5 ± 1.4		
6	5.3 (86.2)	240.9 ± 2.2	215.2 ± 12.4	26.3 ± 3.3	241.5 ± 12.8	9.2 ± 3.3	10.9
7	5.1 (100.9)	170.5 ± 1.9	161.8 ± 9.6	<DL*	161.8 ± 9.6		
8	6.4 (18.7)	331.8 ± 19.2	341.9 ± 15.7	2.6 ± 0.3	344.5 ± 15.7	0.4 ± 0.1	0.8
9	6.5 (9.0)	1,095.9 ± 21.5	1,012.5 ± 29.2	50.5 ± 2.1	1,063 ± 29.3	2.4 ± 0.8	4.6
10	4.9 (110.0)	510.8 ± 7.0	513.7 ± 24.9	<DL*	513.7 ± 24.9		
11	6.5 (13.1)	674.9 ± 15.1	723.8 ± 29.4	<DL*	723.8 ± 29.4		
12	5.6 (69.2)	205.5 ± 2.9	497.5 ± 55.7	14.8 ± 2.6	512.3 ± 55.8	47.9 ± 7.0	7.2
13	5.4 (80.1)	229.4 ± 4.6	618.1 ± 77.3	4.9 ± 0.5	622.9 ± 77.3	27.6 ± 8.6	2.1
14	6.3 (25.7)	302.8 ± 28.1	262.3 ± 7.2	49.7 ± 8.0	312.0 ± 10.8	6.3 ± 1.5	16.4
15	5.3 (86.5)	243.3 ± 4.0	247.4 ± 7.0	<DL*	247.4 ± 7.0		
16	5.0 (107.8)	187.5 ± 7.7	158.4 ± 2.6	<DL*	158.4 ± 2.6		
17	6.3 (24.1)	548.3 ± 5.6	506.5 ± 16.6	<DL*	506.5 ± 16.6		
18	6.5 (9.0)	3.09 ± 0.03	3.8 ± 0.4	<DL*	3.8 ± 0.4		
19	5.6 (70.6)	561.1 ± 11.2	620.1 ± 11.8	18.5 ± 1.9	638.6 ± 12.0	2.9 ± 0.3	3.3
20	6.6 (3.9)	860.9 ± 5.6	820.3 ± 8.2	122.4 ± 13.0	942.7 ± 15.4	5.1 ± 0.8	14.2
21	6.4 (20.2)	58.9 ± 1.6	59.0 ± 0.6	<DL*	59.0 ± 0.6		
22	6.3 (23.7)	47.1 ± 1.3	40.8 ± 3.8	1.5 ± 0.2	42.4 ± 3.8	4.4 ± 0.5	3.3
23	6.4 (20.2)	384.1 ± 11.5	361.7 ± 15.0	3.6 ± 0.3	365.3 ± 15.0	0.5 ± 0.1	0.9
24	5.8 (53.2)	11.9 ± 0.8	14.3 ± 1.1	<DL*	14.3 ± 1.1		

[a]DL: 0.5 ng/g Cr(VI) in sample. DL*: sample is strongly reducing. The entire spiked ^{53}Cr(VI) was lost during extraction, and no Cr(VI) peak was observed in the chromatogram. The lost ^{53}Cr isotope from the ^{53}Cr(VI) was found to be present in the acid digest.

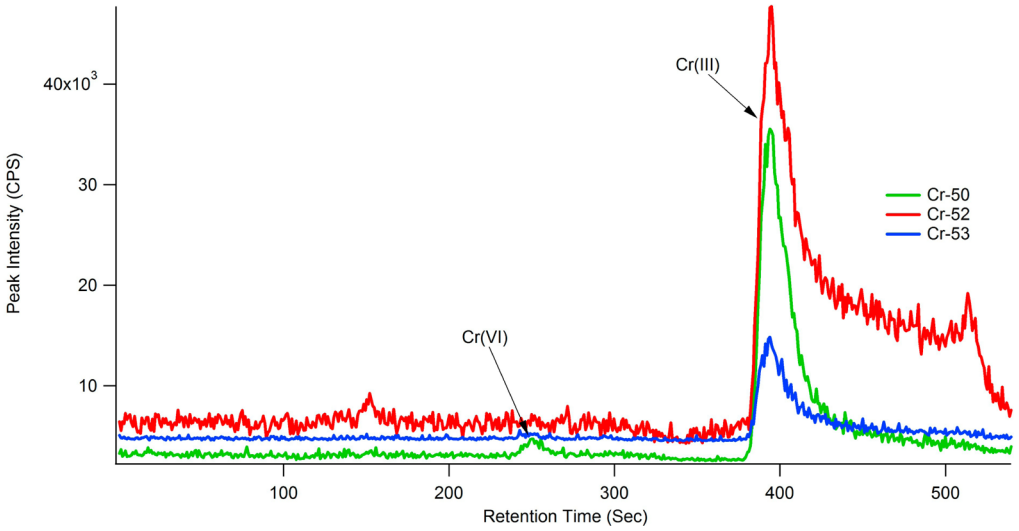

Figure 4. IC-ICP-MS chromatogram for sample 21. Flow rate: 1.0 mL/min with gradient elution. Eluent: A = 0.06 M HNO_3, pH = 9.3; B = 0.06 M HNO_3, pH 1.2. Column: PRP-X100 anion exchange (150 mm × 4.6 mm, 10 μm), from Hamilton.

half of the total chromium concentration measured by IDMS in the specimen. This level of concentration discrepancy may create significant dosing problems for the physicians in the clinic, even potentially harmful therapies. It also underscores the need for reliable, standard total elemental and speciated testing to ensure product quality and safety for supplements, especially for reactive species like Cr(VI) and Cr(III).

Hexavalent Chromium Analysis by SIDMS. The basic application of SIDMS depends on some fundamental operations: enriched stable speciated isotopic spike preparation and calibration and/or purchase of enriched stable isotopic spike analogue; sample collection and sample spiking; sample species and spike species equilibration; sample extraction; species separation; isotope ratio measurements of each speciated component; determination of species concentrations and mathematical deconvolution of species transformations. The method is a direct mathematical solution that does not use traditional calibration curves. Species transformations do not produce traditional bias in SIDMS but are calculated and corrected for in the mathematical protocol specified in EPA

Method 6800 from the time of spiking with enriched isotopic tags. SIDMS equations that are used for Cr(III) to Cr(VI) described in this study were described in previous publications and in EPA Method 6800 update V. The equations applicable to this paper assume that all the chromium species extracted in alkaline solution are Cr(VI) and no Cr(III) is present in the alkaline extract solution based on phase diagrams and previous measurements.[15]

In order to perform SIDMS analyses, samples were double spiked with isotope enriched ^{50}Cr(III) and ^{53}Cr(VI) and extracted in three replicates, and each replicate was analyzed four times to permit statistical evaluation of the samples (n = 3). Just before analysis, sample extracts were diluted 20- to 50-fold in DDI water. After analyses, raw data were exported as CSV file to Microsoft Excel to calculate isotope ratios for each speciated component. Dead time and mass bias corrections for Cr(VI) and Cr(III) data were done by calculating chromium isotope ratios, 50/52 and 53/52. The data acquisition and processing procedures, as well as dead time and mass bias correction procedures, have been reported elsewhere.[19,27−29] As Cr(III) was not present in the alkaline extract (pH 12), it was not detected in IC-ICP-MS measurements. Therefore, only the isotope ratios for the Cr(VI) peak, $R^{VI}_{50/52}$ and $R^{VI}_{53/52}$, were considered and SIDMS calculations were performed to calculate the concentration of Cr(VI), where $R^{VI}_{50/52}$ and $R^{VI}_{53/52}$ are the isotope ratios of ^{50}Cr to ^{52}Cr and ^{53}Cr to ^{52}Cr in Cr(VI), respectively. The final concentration of Cr(VI) in the samples (in μg/g) and the percent transformation of Cr(III) to Cr(VI) during extraction are summarized in Table 4.

It is observed in Table 4 that 13 out of the 24 chromium supplement samples analyzed during this study contain Cr(VI) in a range from 1.5 ± 0.2 μg/g (sample 22) to 122.4 ± 13.0 μg/g (sample 20). Eleven other samples contain Cr(VI) at less than the detection limit (DL = 0.0028 μg/g) or these products have no detectable Cr(VI). These 11 samples contained a highly reducing matrix which effectively transformed all the ^{53}Cr(VI) spike added before extraction to ^{53}Cr(III), during extraction, and precipitated it out as an insoluble chromium compound. Therefore, no Cr(VI) species was detected during IC-ICP-MS analysis. Figure 4 shows a typical chromatogram obtained from one of these nine samples (sample 21).

Hexavalent Chromium Analysis by EPA Method 7196A. Four raw material samples were extracted by Method 3060A and then were analyzed using Method 7196A. The results from this analysis along with SIDMS and IC-ICP-MS (calibration curve) are shown in Table 5. It can be observed from Table 5 that the results from each of the samples were statistically different in different mode of analysis. In all the samples, the Cr(VI) concentration was very small by using Method 7196A, whereas the concentrations by using the IC-ICP-MS calibration curve were higher than the SIDMS results in most of the cases. The results produced by the IC-ICP-MS method were not corrected for conversion from Cr(III) to Cr(VI) during extraction or measurement steps, whereas the results produced by SIDMS were corrected for those conversions. Method 7196A results verified its weakness in proper quantification compared with SIDMS. The color change or reaction with DPC could be affected by the presence of other ingredients within the supplements. Also, the extracts from Method 3060A already had a colored tint to most of the samples. Figure 5 is a photograph showing the coloration of some of the extracted samples using Method 3060A.

Table 5. Comparison of Cr(VI) Values in Dietary Supplement Raw Materials Using Three Detection Techniques[a]

sample	SIDMS (μg/g)	IC-ICP-MS [calibration curve] (μg/g)	EPA Method 7196A (μg/g)
RM1	63 ± 2	155.54 ± 13.72	0.8796
RM2	<DL	545.08 ± 49.96	3.2644
RM3	161 ± 14	143.62 ± 14.30	0.8491
RM4	4,683 ± 226	1,196.83 ± 71.59	14.2806

[a]Uncertainties are at 95% CL with n = 3.

Figure 5. Photograph displaying the color of Method 3060A extracts in some dietary supplements.

Mass Balance Study Using External Calibration Curve and SIDMS Protocols. Two samples were extracted and digested according to the mass balance study protocol and were analyzed using IC-ICP-MS and ICP-MS. The results are reported in Table 6. The sum of the Cr(VI) and Cr(III) for one sample was statistically indistinguishable from that of the total chromium determined by IDMS protocol. On the other hand, the sum of Cr(VI) and Cr(III) for the other sample was statistically different, but within 10% of the total chromium determined by IDMS protocol. From these results, it can be concluded that the mass balance study can be performed using the current protocol based on species-specific isotope dilution mass spectrometry (SSIDMS) protocol by fractionation of Cr(VI) and Cr(III) using three advanced EPA methods (Method 3060A, Method 3052, and Method 6800).

After performing the SIDMS sample preparation protocols on all the samples, the alkaline extracts and the acidic digests were analyzed by IC-ICP-MS and ICP-MS, respectively, and the resulting data were exported in Microsoft Excel compatible format for further manipulations. The final results are shown in Table 4. From Table 4 it can be observed that mass balance was obtained in twenty-one out of twenty-four samples. The summation of Cr(VI) and Cr(III) values were statistically indistinguishable from the total chromium determined by IDMS analysis at their 95% CI. Mass balance could not be achieved for three samples. These three samples were from the same manufacturer but from different batches and product lines. In all three of these samples, a large amount of Cr(III) converted to Cr(VI) during extraction using EPA Method 3060A. Some of the spiked ^{50}Cr(III) was converted to ^{50}Cr(VI) and extracted into the alkaline solution phase as Cr(VI). Some of the spiked ^{50}Cr(III) was present as ^{50}Cr(III) in the alkaline phase, which can be seen in Figure 6 (sample 12). Because of

Table 6. Mass Balance Study for Trivalent and Hexavalent Chromium in Dietary Supplements with External Calibration Protocol Using IC-ICP-MS[a]

		Mass Balance Study using External Calibration Technique (μg/g)		
sample ID	total Cr by IDMS (μg/g)	trivalent Cr	hexavalent Cr	total Cr
9	1095.9 ± 21.5	1,133.4 ± 57.9	73.1 ± 9.2	1,206.5 ± 58.6
12	205.5 ± 2.9	198.1 ± 9.5	4.2 ± 0.5	202.3 ± 9.5

[a]Uncertainties are at 95% CL, n = 3.

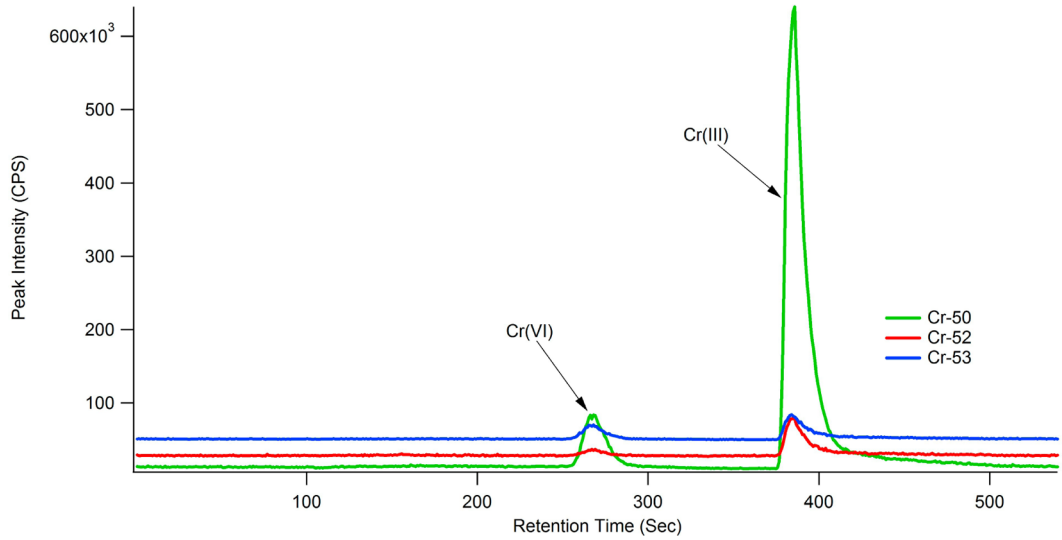

Figure 6. IC-ICP-MS chromatogram for sample 12. Flow rate: 1.0 mL/min with gradient elution. Eluent: A = 0.06 M HNO_3, pH = 9.3; B = 0.06 M HNO_3, pH 1.2. Column: PRP-X100 anion exchange (150 mm × 4.6 mm, 10 μm), from Hamilton.

these losses of the spiked ^{50}Cr(III) in the residue phase, the measured isotopic ratio of 50/52 in the acidic phase, which contains the Cr(III), was less than the expected true isotope ratio. Therefore, the negatively biased isotope ratio produced positively biased concentration for Cr(III). When the spiked isotopically labeled species are lost completely during extraction and/or not in equilibrium with the native species, determination of true species concentrations in the extracts is not possible using isotope dilution metrology. The primary assumption in isotope dilution analysis is that the spiked isotope is in complete equilibrium with the native isotopes. This appears, at the present time, to be occurring to specific ingredients unique to one manufacturer. These findings suggest that review of formulation ingredients, responsible for causing or preventing the stability of chromium species, should be identified and evaluated in order to prevent formation of Cr(VI) in dietary supplements as a result of steps during formulation and production. A study of incipient, content, and formulation method is warranted in a follow-up research.

Being able to compare the mass balance data to the serving size and the recommended daily dose is also important for making conclusions about health effects and for the effective utilization of dietary supplements. Table 7 shows recommended daily doses for the supplements in this study. This table provides the calculated amount of Cr(VI) that would have been consumed daily for each supplement with Cr(VI) content, based on our analysis.

With the heightened health awareness of Cr(VI) and specific regulations and limits, in California, ingestion of dietary supplements containing Cr(VI) may adversely affect public health and could result in legal action especially when the labels do not list the contents accurately. Hence, there is a compelling

Table 7. Amount of Hexavalent Chromium per Serving Size of Select Supplements[a]

sample ID	serving size/day	mass of serving size (g)	Cr(VI) concn (μg/g)	Cr(VI) daily dose (μg)
1	1 tablet	0.269	26.4 ± 2.5	7.10
2	1 capsule	0.283	<DL	
3	1 caplet	1.083	17.0 ± 2.3	18.41
4	1 caplet	1.397	2.6 ± 0.5	3.91
5	1 caplet	1.350	<DL	
6	1 caplet	0.866	26.3 ± 3.3	22.78
7	1 tablet	1.159	<DL	
8	1 capsule	0.582	2.6 ± 0.3	1.51
9	1 tablet	0.446	50.5 ± 2.1	22.52
10	1 tablet	0.432	<DL	
11	1 capsule	0.402	<DL	
12	1 caplet	1.044	14.8 ± 2.6	15.45
13	1 caplet	1.040	4.9 ± 0.5	5.10
14	1 caplet	0.866	49.7 ± 8.0	43.04
15	1 caplet	1.132	<DL	
16	1 tablet	1.145	<DL	
17	1 tablet	0.439	<DL	
18	30 mL	32.785	<DL	
19	1 caplet	0.435	18.5 ± 1.9	8.05
20	1 tablet	0.484	122.4 ± 13.0	59.24
21	1 tablet	1.344	<DL	
22	1 caplet	1.411	1.5 ± 0.2	2.12
23	1 capsule	0.603	3.6 ± 0.3	2.17
24	1 tablet	0.649	<DL	

[a]Uncertainties are at 95% CL, n = 3.

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need to use more rigorous, accurate finished product testing under current good manufacturing practices (cGMP) following more effective quality assurance (QA) protocols. Speciated measurement for many toxins and/or carcinogens, such as Cr(VI), must be a part of standard QA.

The procedure applied for the dietary supplements in this paper can be expanded to other areas where the analyte exists in complex matrices such as foods and other species. Chromium speciation is important in various other matrices, such as foods, food additives, and substitutes. Although this research was focused on the analytical aspect of chromium species in dietary supplements, it can also serve other studies on the health effects of each of the chromium species. Even though carcinogenicity of Cr(VI) through the oral route of exposure is still debated, having accurate testing, robust quality assurance, and proper regulatory policies that will ensure that the form of chromium in the final supplement product is Cr(III) and not Cr(VI) is an important need for public safety.

This paper demonstrates the utility of the SIDMS protocol of the EPA Method 6800 as an analytical tool as well as its application as a powerful QA tool in testing because it can more accurately assess the chromium-containing formulations that have the highest chance to be ingested as or metabolically converted to Cr(VI), regardless of the speciated form chromium may be when it is received from raw material suppliers and in the final products. The measurement of total chromium is not adequate to assess the nutritional and or toxicological bases of chromium containing supplements.

The dietary supplement market in the United States is the largest segment in the world, reaching $28 billion in 2010.[30] The popularity of these products and the different health implications from trivalent and hexavalent chromium make speciation analysis with the mass balance results important for companies and consumers. Considering the results of our study and increasing number of food/supplement contamination events, we believe the supplement producers must voluntarily evaluate their entire supply chain, starting with the incoming ingredients, and realize the importance of proper sample preparation and analysis techniques to test their products to meeting labeling regulations and to ensure consistent product purity and quality.

AUTHOR INFORMATION

Corresponding Author

*Tel: +1 (412) 223-2096. E-mail: rahman@sidms.com.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Office of Dietary Supplements: National Institute of Health. *Dietary Supplement Fact Sheet: Chromium*. http://ods.od.nih.gov/factsheets/chromium (accessed September 12, 2011).

(2) Institute of Medicine, Food and Nutrition Board. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; National Academy Press: Washington, DC, 2001.

(3) Environmental Protection Agency. *Toxicological Review of Trivalent Chromium*. http://www.epa.gov/ncea/iris/toxreviews/0028tr.pdf (accessed September 12, 2011).

(4) Occupational Safety & Health Administration. *Chromium(VI) (Hexavalent Chromium)*. http://www.osha.gov/dts/chemicalsampling/data/CH_228697.html (accessed September 21, 2011).

(5) American Cancer Society. *Known and Probable Human Carcinogens*. http://www.cancer.org/Cancer/CancerCauses/OtherCarcinogens/GeneralInformationaboutCarcinogens/known-and-probable-human-carcinogens (accessed September 21, 2011).

(6) Office of Environmental Health Hazard Assessment. *Proposition 65*. http://oehha.ca.gov/prop65.html (accessed September 12, 2011).

(7) Nagourney, S. J.; Wilson, S. A.; Buckley, B.; Kingston, H. M.; Yang, S. Y.; Long, S. E. Development of a standard reference material for Cr(VI) in contaminated soil. *J. Anal. At. Spectrom.* **2008**, *23*, 1550−1554.

(8) EPA Method 7196A, Chromium, Hexavalent (Colorometric). In *Solid Waste manual—846*; US Government Printing Office: Washington, DC, 1992.

(9) EPA Method 7199, Determination of Hexavalent Chromium in Drinking Water, Groundwater and Industrial Water Effluents by Ion Chromatography. In *Solid Waste manual—846*; US Government Printing Office: Washington, DC, 1996.

(10) Method 3052, Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. In *Solid Waste manual—846*; US Government Printing Office: Washington, DC, 1996.

(11) Method 3060A, Alkaline Digestion for Hexavalent Chromium. In *Solid Waste Manual—846*; US Government Printing Office: Washington, DC, 1996.

(12) Method 6800, Elemental and Speciated Isotope Dilution Mass Spectrometry. In *Solid Waste Manual—SW-846, Update IV*; US Government Printing Office: Washington, DC (RCRA Update V), 2012.

(13) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. Accuracy in species analysis: Speciated isotope dilution mass spectrometry (SIDMS) exemplified by the evaluation of chromium species. *Spectrochim. Acta, Part B* **1998**, *53*, 299−309.

(14) Huo, D. PhD Dissertation. The development of methods in Speciated isotope dilution mass spectrometry (SIDMS) for elemental speciation of chromium in environmental samples using LC-ICP-MS. Duquesne University, Pittsburgh, PA, USA, 1999.

(15) Huo, D.; Skip Kingston, H. M.; Larget, B. Application of Isotope Dilution in Elemental Speciation. Chapter 10 in *Elemental speciation: new approaches for trace element analysis*, Vol. *33*, Comprehensive analytical chemistry; Caruso, J. A., Sutton, K. L., Ackley, K. L., Eds.; Elsevier: New York, 2000.

(16) Huo, D.; Kingston, H. M. Correction of species transformations in the analysis of Cr(VI) in solid environmental samples using Speciated isotope dilution mass spectrometry. *Anal. Chem.* **2000**, *72*, 5047−5054.

(17) Huo, D.; Lu, Y.; Kingston, H. M. Determination and correction of analytical biases and study on chemical mechanisms in the analysis of Cr(VI) in soil samples using EPA protocols. *Environ. Sci. Technol.* **1998**, *32*, 3418−3423.

(18) Rahman, G. M. M.; Kingston, H. M. Application of Speciated isotope dilution mass spectrometry to evaluate extraction methods for determining mercury speciation in soils and sediments. *Anal. Chem.* **2004**, *76*, 3548−3555.

(19) Rahman, G. M. M. PhD Dissertation. Development, research and validation of environmental speciation methods: Evaluation by Speciated isotope dilution mass spectrometry in mercury and

chromium speciation analysis. Duquesne University: Pittsburgh, PA, USA, 2004.
(20) Yang, L.; Ciceri, E.; Mester, Z.; Sturgeon, R. E. Application of double-spike isotope dilution for the accurate determination of Cr(III), Cr(VI) and total Cr in yeast. *Anal. Bioanal. Chem.* **2006**, *386*, 1673−1680.
(21) Meija, J.; Yang, L.; Caruso, J. A.; Mester, Z. Calculations of double spike isotope dilution results revisited. *J. Anal. At. Spectrom.* **2006**, *21*, 1294−1297.
(22) Monperrus, M.; Rodriguez Gonzalez, P.; Amouroux, D.; Alonso, J. I. G.; Donard, O. F. X. Evaluating the potential and limitations of double spiking species specific isotope dilution analysis for the accurate quantification of mercury species in different environmental matrices. *Anal. Bioanal. Chem.* **2008**, *390*, 655−666.
(23) Point, D.; Davis, W. C.; Alonso, J. I. G.; Monperrus, M.; Christopher, S. J.; Donard, O. F. X.; Becker, P. R.; Wise, S. A. Simultaneous determination of inorganic mercury, methylmercury, and total mercury concentrations in cryogenic fresh-frozen and freeze-dried biological reference materials. *Anal. Bioanal. Chem.* **2007**, *389*, 787−798.
(24) Rodriguez Gonzalez, P.; Monperrus, M.; Alonso, J. I. G.; Amouroux, D.; Donard, O. F. X. Comparison of different numerical approaches for multiple spiking species-specific isotope dilution analysis exemplified by the determination of butyltin species in sediments. *J. Anal. At. Spectrom.* **2007**, *22*, 1373.
(25) Garcia-Alonso, J. I. Determination of fission products and actinides by inductively coupled plasma mass spectrometry using isotope dilution analysis: A study of random and systematic errors. *Anal. Chim. Acta* **1995**, *312*, 57−78.
(26) Gurleyuk, H.; Wallschlager, D. Determination of chromium (III) and Chromium (VI) using suppressed ion chromatography inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2001**, *16*, 926−930.
(27) Taylor, H. E. In *Inductively coupled plasma mass spectrometry: practices and techniques*; Academic: San Diego, 2001; p 48.
(28) Elemental VG. *VG Plasmaquad system manual*; VG Elemental Ltd: Winsford, England, 1989.
(29) Jarvice, K. E.; Gray, A. L.; Houk, R. S. In *Handbook of inductively coupled plasma mass spectrometry*; Blackie Academic & Professional: London, 1992; p 315.
(30) BusinessWire. *Research and Markets: Nutritional and Dietary Supplements Market in the US—Opportunities and Future Outlook*. http://www.businesswire.com/news/home/20110822005646/en/Research-Markets-Nutritional-Dietary-Supplements-Market--, 2010 (accessed October 28, 2011).